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- https://www.who.int/mediacentre/news/release/2008/pr48/en/
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- https://ods.od.nih.gov/factsheets/Molybdenum-Consumer/
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APPENDIX-1

ALPHA
LABS & TECHNOLOGIES
FOOD / WATER / SOIL TESTING
An ISO 17025 (NABL) Accredited & ISO 9001 Certified Laboratory

TC - 5573

281-C, Thadagam Road, Amutha Surabi Upstairs, Opp. Avila Convent, Venkittapuram, Coimbatore-641025.
Tel : 0422 - 2441499 Mobile: 96294 40642 E-mail : alphalabtech@gmail.com www.alphalabtech.in

TEST REPORT

REPORT NO:	ALT/TRE/II/1032 B	REPORT DATE:	30.01.2021

Customer Name & Address	**M/s. AKHILA.C AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN, COIMBATORE.**		
Sample Description:	IRaRW		
Sample Code no:	ALT/FA/1032 B	Sampled By:	Customer
Sample Collected Date	28.01.2021	Analysis started on	28.01.2021
Sample received on	28.01.2021	Analysis completed on	30.01.02021

RESULTS OF THE ANALYSIS

S.NO	TEST PARAMETERS	UNIT	TEST PROTOCOL	RESULT
1.	Moisture	g/100g	AOAC 20th edition 925.10	**98.81**
2.	Total Ash	g/100g	AOAC 20th edition 923.03	**0.03**
3.	Fat	g/100g	AOAC 20th edition 2016 920.85	**0.07**
4.	Total Protein	g/100g	IS 7219	**0.52**
5.	Carbohydrate	g/100g	AOAC 20th edition 986.25 E , IS 1656 Annexure C	**0.57**
6.	Energy	KCals/100g	ALT/SOP/III/4.d/11 Issue no: 1 Issue date: 10.07.2016	**5.05**
7.	Fiber	g/100g	AOAC 20th edition,920.86	**0.07**
8.	Vitamin B6	mg/kg	AOAC Official Method 968.22, 18th Edition	**20.24**
9.	Vitamin B12	mg/kg	AOAC Official Method 968.22, 18th Edition	**BLQ [LOQ-5.0]**

ULR No: TC557320000001987F

*****End of Report*****

S.P. Mohan Raj
Technical Manager
Authorized Signatory

APPENDIX-2A

Antibacterial susceptibility Testing by Kirby-Bauer method for coated earbuds

Antibacterial Susceptibility Testing by Kirby-Bauer method was adopted for the coated ear buds with *Staphylococcus aureus* inoculums. From an agar plate culture, at least three to five well-isolated colonies with the same morphological type are chosen. The growth is transferred into a tube containing 4 to 5 ml of an appropriate broth medium, such as Muller-Hinton broth, by touching the top of each colony with a loop. Until the turbidity is reached or exceeded, the broth culture is incubated at 35^0C. (Usually 2 to 6 hours). To achieve turbidity, the turbidity of the broth culture is modified with sterile saline or broth. As a result, the suspension has a *Staphylococcus aureus* concentration of between 1 and 2 x 10^8 CFU/mL. A sterile cotton swab should be dipped into the corrected suspension within 15 minutes of modifying the turbidity of the inoculum suspension. The swab should be turned around many times and firmly pressed against the interior tube wall above the fluid level. This will clear the swab of extra inoculum. A swab is used to streak the sterile agar surface of a Müller-Hinton agar plate to inoculate the dried surface. To achieve a uniform dispersion of inoculum, streaking is performed two more times with the plate being rotated by around 600rpm each time. The rim is swabbed with agar as a last step. Before applying the drug-impregnated discs, the lid may be left in the jar for 3 to 5 minutes, but no longer than 15 minutes, to allow any excess surface moisture to be absorbed. The coated ear buds and blank were placed and petriplates were placed inversely for complete diffusion and inhibition zones were examined.

APPENDIX-2B

Antibacterial susceptibility Testing for GNP and SNP synthesized using cereals and pulses washed water

Three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as Müeller-Hinton broth. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity (usually 2 to 6 hours). The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain turbidity. This results in a suspension containing approximately 1 to 2 x 10^8 CFU/ml for *E.coli, Staphylococccus aureus, K. pneumoniae and B.cereus.* Approximately, within 15 min after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. The dried surface of a Müller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed. The media was punctured by making a well of 6 mm in diameter and filled with 50 µL of a sample. Further the petriplates were placed inversely for complete diffusion and inhibition zones were examined by measuring the diameter (mm) formed around the well after 24 h incubation at 37°C. The zones were measured by using standard (Hi-Media) scale.

APPENDIX-3

Procedure for MTT assay

The cytotoxicity of the nanoparticles was ascertained by MTT assay 3 - (4, 5 - Dimethylthiazol - 2 - yl) - 2, 5 - diphenyltetrazolium bromide).The IC_{50} value was taken at 24 h. Skin cancer cell line (A375) were grown (1 × 10 5 cells/ well) in a 96 - well plate. The medium was treated with BJAgSo, IBRRWM, IRaRWSoAg, BJWM, WCWM, WCAgSo, BJWW, IBRRW, IRaRW & WCWW. The samples loaded cells were incubated for 24 h. MTT solution (100 µL) was added then incubated for 4 h at 37 °C. The medium was replaced with 50 µL DMSO and again incubated for 10 min to form a formazan solution. The absorption was measured at 620 nm using ELISA multi well plate reader.

APPENDIX-4

Procedure for comet assay

To make electrophoresis buffer (pH 13-14), dissolve 12 g NaOH and 372 mg EDTA in 1 litre distilled water. To make neutralisation buffer (pH 7.2), dissolve 12.11g Tris-Base in 250 mL distilled water. Lysis solution (pH 10) is made by dissolving 146.1g NaCl, 37.2g Na_2 EDTA, and 1.2g Tris HCl in 700mL distilled water. 12 g pelletized NaOH is added and agitated until the salts are completely dissolved in the solution. The pH of the solution was adjusted to 10.0 using 0.1N HCl / NaOH, and it was filtered, sterilised, and stored at room temperature. The aforementioned solution was treated with Triton X-100 (1% in 10 mL) and chilled for 30 - 60 minutes before use. To make ethidium bromide dye, add 5 µl Ethidium bromide stock (10 mg/mL) per 100 mL gel solution for a final concentration of 0.5ug/mL. The cells were processed by trypsining them in a T25 flask and creating a pellet from that. Phosphate buffer saline (PBS) was used to wash the pellets three times. In addition, 1% standard agarose and 1% low melting agarose were made.

For electrophoresis, the standard apparatus model was employed. The gels used in the experiment differ. The microgel electrophoresis system is used in the comet test; gels are put in an 18 × 18 mm area on a totally frosted microslide. On each slide, two samples, each comprising 1000-2000 cells are casted.

Normal agarose (200µl) (1%) was gently deposited onto a totally frosted micro-slide in Phosphate buffer saline (PBS) at 65^{o}C. The slides were covered and placed on an ice pack (5 min). The slides were left open until the gel had dried. At 37^{o}C, a fraction of the cell suspension was combined with agarose in 1:3 ratio. This 100µl mixture was applied to the gel, coated, and allowed to set. The final coating was applied in the same manner. Each cell fraction was produced in the same way. About 16 hours, the slides were submerged in an ice cold lysis solution at 4^{o}C. This was only done after the agarose had solidified. By completing the cell lysis under low illumination conditions, any additional DNA damage was avoided

The experiment was carried out in an electrophoresis tank with slides arranged horizontally. The reservoirs were filled with electrophoresis buffer, and the slides were immersed for 20 minutes. For 15 minutes, electrophoresis was performed at 0.8v/cm. The slides were washed with neutral buffer after electrophoresis. The gel was coated with ethidium bromide, and the slides were covered. A fluorescent microscope fitted with a 365nm excitation filter and a 435nm barrier filter was used to study the DNA at magnifications of 200x and 400x.

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